# Cholecystokinin (CCK) regulates somatostatin secretion through both the CCK-A and CCK-B/gastrin receptors in sheep

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1. Cholecystokinin (CCK) and gastrin both stimulate gastric somatostatin (SOM) secretion *in vitro* and thus have the potential to modulate their direct effects on the parietal cell. However, the relative potencies and the mechanisms of action of CCK and gastrin on SOM secretion *in vivo* have not been determined.

2. The objectives of the present study were to compare the *in vivo* potencies of the sulphated (s) and non-sulphated (ns) forms of gastrin heptadecapeptide (G-17) and CCK octapeptide (CCK-8) on SOM secretion, and to determine the nature of the receptors involved by repeating the studies in the presence of the CCK-A and CCK-B/gastrin receptor antagonists L-364,718 and L-365,260, respectively. All experiments were performed in the chronically cannulated sheep.

3. Dose–response experiments revealed the following potencies for SOM secretion: G-17s = CCK-8s > G-17ns ≫ CCK-8ns. However, based on the plasma levels achieved and a higher metabolic clearance rate (MCR) for CCK, CCK-8s was the most potent.

4. Both the CCK-A and CCK-B/gastrin receptor antagonists suppressed CCK-8s-stimulated SOM output. In contrast, G-17s-stimulated SOM output was inhibited by only the CCK-B/gastrin receptor antagonist.

5. Both receptor antagonists increased basal plasma gastrin and CCK levels.

6. The predominant circulating SOM molecular form after both gastrin and CCK stimulation was SOM-14.

7. In conclusion, the sulphated forms of CCK and gastrin are more potent than the non-sulphated forms. Despite sharing a common biologically active carboxy terminus, CCK stimulates SOM secretion by both the CCK-A and CCK-B/gastrin receptors, while gastrin acts via the CCK-B/gastrin receptor alone. These findings explain in part why CCK is a net inhibitor of gastric acid secretion *in vivo*.

Gastrin and cholecystokinin (CCK) share a common biologically active carboxy terminus (Mutt & Jorpes, 1968). Despite this structural similarity, there is a differential effect by these peptides on acid secretion (Soll, Amirian, Park, Elashoff & Yamada, 1985). *In vitro* CCK and gastrin are equipotent at stimulating parietal cell function (Soll, Amirian, Thomas, Reedy & Elashoff, 1984; Sandvik & Waldum, 1991). However, *in vivo* the net effect of CCK is to reduce acid secretion, and this has been explained by the more potent somatostatin (SOM) stimulatory effect of CCK (Schmidt, Schenk, Nustede, Holst, Folsch & Creutzfeldt, 1994).

A number of studies both *in vivo* and *in vitro* have demonstrated that CCK stimulates SOM secretion by preferentially binding to the CCK-A (type A) receptor. The CCK-A receptor present on D-cells has a greater affinity for sulphated (s) CCK (nearly all circulating CCK is sulphated) than sulphated or non-sulphated (ns) gastrin (about 50% of circulating gastrin is sulphated) (Silvente-Poirot, Dufresne, Vaysse & Fourmy, 1993). The second receptor subtype, the CCK-B/gastrin (type B) receptor, which is present on parietal as well as enterochromaffin-like (ECL) cells (Soll *et al.* 1984; Prinz, Kajimura, Scott, Mercier, Helander & Sachs, 1993) does not distinguish between gastrin and CCK

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whether sulphated or not (Silvente-Poirot *et al.* 1993). Ligand binding evidence suggests that both type A and type B receptors are present on isolated canine fundic D-cells whereas only the type A receptors were found on the isolated antral D-cell (DelValle, Chiba, Park & Yamada, 1993; Park, Takeda, DelValle & Yamada, 1994). Immunohistochemistry of canine stomach confirmed the presence of both receptors in the fundus but the receptors were not localized to the D-cell (Mantyh, Pappas & Vigna, 1994). In the rat the CCK-B/gastrin receptor was detected by immunohistochemistry on D-cells in both the antrum and fundus (Song, Wong, Ohning & Walsh, 1996). Despite the apparent presence of both receptor subtypes on the D-cell, there has been no systematic comparison of the relative potency of the sulphated and non-sulphated forms of both gastrin and CCK in stimulating SOM secretion. The relative contribution of the A and B receptor subtypes as mediators of SOM secretion has also not been determined. On the assumption that the type A receptor was the regulator of gastric SOM, previous studies have only tested the effects of the type A receptor antagonists (Lloyd, Maxwell, Kovacs, Miller & Walsh, 1992*a*; Lloyd, Raybould & Walsh, 1992*b*; DelValle *et al.* 1993).

Somatostatin is the inhibitory mediator of the gastrin–acid regulatory feedback loop (Shulkes, 1994) and it is perhaps not surprising that changes in gastrin and CCK secretion have been reported in association with administration of the CCK-A and CCK-B antagonists. However, the changes have been quite variable both between and within species (Beglinger *et al.* 1992; Konturek, Konturek, & Domschke, 1993; Schmidt *et al.* 1994) and in no single study have the gastrin, CCK and SOM responses been measured, nor have both receptor antagonists been tested.

The development of non-peptide potent and selective CCK receptor antagonists has provided a means by which the role of gastrin and CCK in gastrointestinal function can be investigated. The benzodiazepine derivative L-364,718 (MK-329, devazepide) is highly selective for the CCK-A receptor, and antagonizes CCK-regulated gastrointestinal functions including gastric acid, SOM and pancreatic secretion and gall bladder contraction (Lotti, Pendleton, Gould, Hanson, Chang & Clineschmidt, 1987). A related compound, L-365,260 is selective for the CCK-B/gastrin receptor and has been used to study the role of the CCK-B/gastrin receptor in acid secretion (Lotti & Chang, 1989; Varga, Campbell, Bussjaeger & Solomon, 1993).

The sheep is a particularly good model for understanding the regulation of SOM as, in contrast to humans (Verhulst *et al.* 1994), changes can be measured in plasma rather than gastric juice. Changes in circulating SOM can be measured in response to gastrin and CCK, and these increases are generally independent of changes in gastric acidity (Shulkes & Read, 1991). For this reason the direct effects of gastrin and CCK on D-cell function can be effectively studied.

The objective of the present study was to compare the potencies of the sulphated and non-sulphated forms of both gastrin and CCK on SOM secretion. The nature of the receptors involved were determined by repeating the studies in the presence of the type A and type B receptor antagonists. In addition changes in plasma gastrin and plasma CCK concentrations in response to L-364,718 and L-365,260 were measured to determine the interactions of the components of the somatostatin–gastrin regulatory loop.

## METHODS

### Animal preparation

Merino × Corriedale sheep, ranging from 35 to 40 kg body weight, were administered a local anaesthetic (2 ml Xylocaine, 1%) in the skin surrounding the jugular vein. A hypodermic needle was inserted into the jugular vein, and a cannula passed through the needle and into the vein directed toward the head (up). The same procedure was followed for the contralateral jugular vein except that the cannula was directed toward the heart (down). The down cannulae were used for drug and peptide infusions and the up cannulae were used for blood collection. A recovery period of 24 h was allowed. Sheep were housed in metabolism cages for the duration of the experiments. Gastric juice was sampled in some of the sheep. To insert the gastric cannula anaesthesia was induced with intravenous pentothal (1 g) and maintained with 2% fluothane in oxygen. A right subcostal incision was made for insertion of a catheter (polyvinyl chloride, 10 mm i.d., 1·5 mm o.d.) through a purse-string suture into the antral region of the abomasum. The cannula was secured by tightening of the suture and exteriorized through a separate stab incision in the flank. Muscle and fascia was closed with continuous nylon and the skin by a continuous silk suture (Shulkes & Read, 1991). The operated sheep were given three daily doses of antibiotic and allowed 3–7 days to recover from surgery during which time food intakes returned to levels observed prior to surgery. All animal experiments were approved by the Austin and Repatriation Medical Centre Animal Ethics Committee (approval no., 95/030)

### Experimental design

The sheep were fasted for 12–18 h before each experiment. Experiments were performed in random order.

### Agonist studies

**Gastrin and CCK infusions.** Dose–response curves to CCK and gastrin peptides were performed to determine the dose-dependent stimulation of SOM secretion. The peptides used were the sulphated and non-sulphated forms of CCK octapeptide (CCK-8s and CCK-8ns, respectively) and gastrin heptadecapeptide (G-17s and G-17ns, respectively) (all from Bachem, CA, USA) and tetragastrin (Research Plus, NJ, USA). There was a 48 h recovery period between infusions of each peptide. The peptide was dissolved in 0·9% w/v NaCl saline–0·1% w/v bovine serum albumin (BSA) and doses of 16,. 50 and 150 pmol $kg^{-1}$ $h^{-1}$ were sequentially infused via the down cannula using a Sage syringe infusion pump (Sage Instruments, Orion Research Inc., MA, USA), at a rate of 40 ml $h^{-1}$ for 30 min. Between each dose there was a 45 min break before the next dose was administered. Blood samples (5 ml) were collected via the up cannula every 15 min. Blood samples were also collected 30 min prior to the beginning of the first infusion, to

establish basal levels. The blood was centrifuged and the plasma frozen for subsequent SOM and gastrin assays.

**G-17s and CCK-8s infusions.** The first series of experiments were performed to determine the relative stimulatory effect of CCK and gastrin on SOM secretion. As presented in Results, the sulphated forms of CCK and gastrin both stimulated SOM secretion at 150 pmol $kg^{-1}$ $h^{-1}$ so subsequent experiments were performed with these two peptides. G-17s or CCK-8s (150 pmol $kg^{-1}$ $h^{-1}$) was infused for 60 min at a rate of 20 ml $h^{-1}$. Blood samples (5 ml) were collected every 15 min, during a 30 min pre-infusion (basal) period, during infusion of peptide and during a 30 min post-infusion period. After 48 h the experiment was repeated using the other peptide.

### Antagonist studies

These experiments determined the roles of the CCK-A and CCK-B/gastrin receptors as mediators of CCK- and gastrin-stimulated SOM secretion.

**L-364,718 and L-365,260 alone.** To assess whether L-364,718 (CCK-A receptor antagonist), L-365,260 (CCK-B/gastrin receptor antagonist) or vehicle dimethylsulphoxide (DMSO)–polyethyleneglycol 400 (PEG 400) had an effect on SOM secretion the antagonists and vehicle alone on plasma SOM concentration were first tested. Due to limited solubility the antagonists were dissolved in DMSO–PEG 400 (9 : 1). Two control experiments were performed. In the first control experiment the vehicle alone was administered via a 5 ml bolus injection into the heart jugular vein followed by a 60 min 0·9% saline–0·1% BSA infusion. In subsequent experiments, and on separate occasions, L-364,718 and L-365,260 (Merck Research Laboratories, Rahway, NJ, USA) were administered instead of the vehicle alone. Blood samples and gastric juice (3–5 ml) were collected every 15 min (Shulkes & Read 1991). All blood samples were centrifuged, and plasma extracted for SOM and gastrin assays.

**L-364,718 or L-365,260 followed by G-17s and CCK-8s infusions.** These experiments were similar to the agonist experiments involving the G-17s and CCK-8s infusions, except that 30 min preceding G-17s or CCK-8s infusions, the antagonist was administered as a bolus injection. L-364,718 (1 mg $kg^{-1}$) or L-365,260 (3 mg $kg^{-1}$) was dissolved in DMSO/PEG 400 (9 : 1) and administered over 5 min by a 5 ml bolus intravenous injection into the jugular vein cannula directed toward the heart. Blood samples and gastric juice were collected every 15 min. After 48 h the experiments were repeated using the other antagonist.

**L-364,718 plus L-365,260 followed by CCK-8s infusion.** To determine whether CCK-8s was acting via both the type A and B receptors, the antagonists were administered together as a 5 ml bolus followed 30 min later by the CCK-8s infusion.

### Reverse phase HPLC: predominant somatostatin molecular form

To determine the proportions of SOM-14 and SOM-28 stimulated by G-17s and CCK-8s, plasma samples during the infusion of either CCK or gastrin and from the antagonist experiments were pooled and purified using Sep-Pak C18 cartridges (Waters, Millipore Corporation, MA, USA). SOM-14 and SOM-28 standards (1 pmol), and purified plasma samples, on separate occasions, were loaded onto a C18 uBondapak column (Waters Associates, USA) and separated using a 22 min gradient from 28–32% ACN–0·05% TFA. The flow rate was set at 3 ml $min^{-1}$ and forty-four 0·5 min fractions were collected. Two hundred microlitres of each standard fraction, and the entire 1·5 ml volume from each sample fraction, were dried down under air. Standards and samples were assayed under normal SOM radioimmunoassay conditions.

### Radioimmunoassay (RIA)

**Somatostatin (SOM).** Plasma SOM concentration was measured in ethanol extracted plasma as described previously (Reasbeck, Burns & Shulkes, 1988). One millilitre of plasma was mixed with 2 ml absolute ethanol and the coagulated protein removed by centrifugation. The ethanol extracts were then divided into 1 ml aliquots, evaporated to dryness and stored at −20 °C until assayed. Antiserum 8402 which detects SOM-14 and SOM-28 to an equal extent was used. [$^{125}$I]-Tyr-SOM-14 was prepared using the chloramine T method and purified by reverse phase HPLC. Charcoal stripped ovine extracted plasma (CSOEP) tubes were used for the SOM-14 standard curves. The $ID_{50}$ was 12 fmol $ml^{-1}$ and the intra- and interassay coefficients of variation were less than 5 and 12%, respectively.

**Gastrin.** Plasma gastrin concentration was assayed directly using 50 $\mu$l of plasma (Shulkes & Read, 1991). The plasma samples were incubated at 4 °C in duplicate with the $^{125}$I-human G-17ns label and antiserum 1296. Antiserum 1296 recognizes all carboxy terminal fragments larger than the pentapeptide and measure G-17s and G-17ns identically. G-17ns was used for generating the standard curve. The $ID_{50}$ was 1 fmol $ml^{-1}$ and the intra- and interassay coefficients of variation were less than 2 and 11%, respectively.

**Cholecystokinin (CCK).** Plasma CCK concentration was measured in ethanol extracted plasma as described for the SOM RIA. Antiserum 92128 and $^{125}$I-Bolton-Hunter-CCK-8 label (Amersham International) were used in the assay. The antiserum is specific for CCK amide with negligible cross-reactivity to gastrin amide or gly extended forms of gastrin and CCK. CSOEP tubes were used for the CCK-8ns standard curve. The $ID_{50}$ was 0·61 ± 0·06 fmol $ml^{-1}$ and the intra- and interassay coefficients of variation were 7 and < 14%, respectively.

### Calculations and statistical analysis

The metabolic clearance rate (MCR) for CCK-8s and G-17s was determined as described by Ciccotosto & Shulkes (1992). MCR was calculated as the dose divided by the equilibrium concentration, where the dose is the infusate concentration (measured by RIA) times the infusion rate (20 ml $h^{-1}$), and the equilibrium concentration is the mean plasma levels at plateau minus mean basal levels.

Results are expressed as the means ± S.E.M.; $n$ is the number of animals. Statistical comparisons between more than two groups were made by one-way analysis of variance followed by the Student–Newman–Keuls method. Statistical comparisons of means between two groups were made using an unpaired $t$ test. $P < 0·05$ was considered significantly different. The integrated SOM response was calculated as the area under the plasma SOM concentration–time curve as described by Richardson, Walsh, Hicks & Fordtran (1976).

## RESULTS

### Agonist studies

**Gastrin and CCK infusions.** The effects of G-17s, G-17ns and tetragastrin on SOM secretion are represented as the integrated SOM outputs in Fig. 1. At doses of 16 and 50 pmol $kg^{-1}$ $h^{-1}$ the effects of G-17s, G-17ns and tetra-

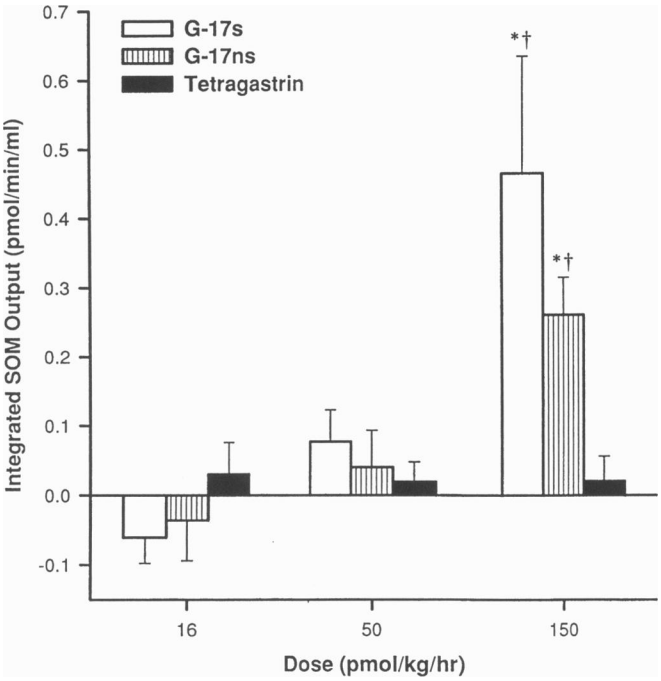


**Figure 1. Integrated SOM output in response to the I.V. infusion of G-17s, G-17ns and tetragastrin**
The integrated output was calculated as the SOM output during the 30 min infusion at each dose. Columns and error bars indicate means ± S.E.M., respectively, $n = 5$, $*P < 0{\cdot}05$ *vs.* basal output, $\dagger P < 0{\cdot}05$ *vs.* tetragastrin.

gastrin were not significantly different from each other or from baseline ($P > 0{\cdot}05$). However, at 150 pmol $kg^{-1}$ $h^{-1}$, G-17s and G-17ns caused a significant stimulatory effect on SOM output compared with baseline ($P < 0{\cdot}05$). The response to G-17s was greater than that of G-17ns but not significantly so ($P > 0{\cdot}05$). Tetragastrin did not change SOM output even at the highest dose. Therefore a dose of 150 pmol $kg^{-1}$ $h^{-1}$ of G-17s was used in subsequent experiments.

Plasma SOM concentration was unchanged by CCK-8s and CCK-8ns infusions at 16 and 50 pmol $kg^{-1}$ $h^{-1}$. At 150 pmol $kg^{-1}$ $h^{-1}$ CCK-8s increased plasma SOM concentration from a basal level of 25 ± 7 pmol $l^{-1}$ to a peak of 51 ± 9 pmol $l^{-1}$. In contrast, CCK-8ns had no effect on SOM secretion at doses of 16, 50 and 150 pmol $kg^{-1}$ $h^{-1}$, with mean values of 17 ± 3, 18 ± 3 and 18 ± 2 pmol $l^{-1}$, respectively, compared with a control value of 23 ± 3 pmol $l^{-1}$. The integrated SOM output for CCK-8s at 150 pmol $kg^{-1}$ $h^{-1}$ (0·33 ± 0·11 pmol $min^{-1}$ $ml^{-1}$) was significantly greater than the SOM output in response to CCK-8ns (−0·05 ± 0·06 pmol $min^{-1}$ $ml^{-1}$) ($P < 0{\cdot}05$) (Fig. 2). A dose of 150 pmol $kg^{-1}$ $h^{-1}$ of CCK-8s was used in the following experiments.

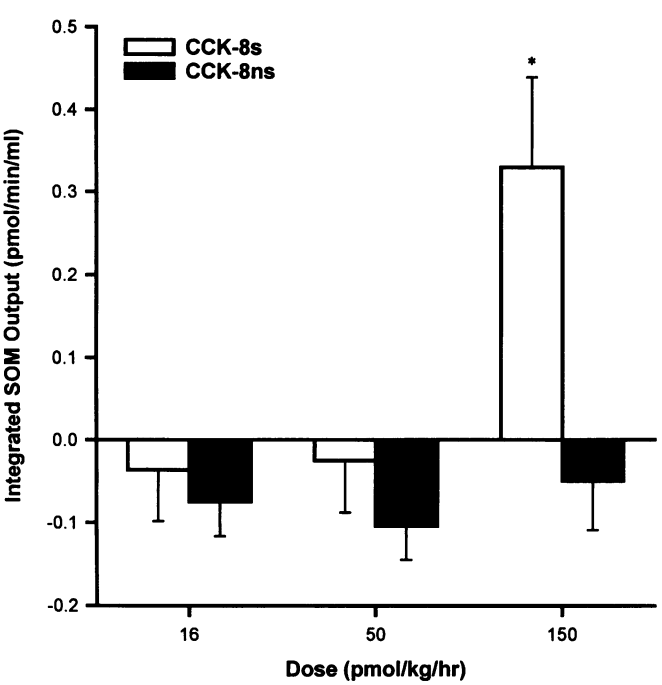


**Figure 2. Integrated SOM output in response to the I.V. infusion of CCK-8s and CCK-8ns**
The integrated output was calculated as the SOM output during the 30 min infusion at each dose. Columns and error bars indicate means ± S.E.M., respectively, $n = 7$, $*P < 0{\cdot}05$ *vs.* basal output.

**Table 1. MCR of G-17s and CCK-8s (150 pmol kg$^{-1}$ h$^{-1}$)**

| Peptide | Measured dose (pmol kg h$^{-1}$) | Plasma levels: Basal (pmol l$^{-1}$) | Plasma levels: Post-infusion (pmol l$^{-1}$) | Plasma levels: Equilibrium (pmol l$^{-1}$) | MCR (ml kg$^{-1}$ min$^{-1}$) |
|---|---|---|---|---|---|
| G-17s | 141 ± 9 | 46 ± 20 | 116 ± 29 | 313 ± 57 | 9·6 ± 1·7 |
| CCK-8s | 134 ± 23 | 6 ± 0·3 | 7 ± 3 | 34 ± 7 | 92·1 ± 29·7 |

**G-17s and CCK-8s infusions.** G-17s and CCK-8s were infused at 150 pmol kg$^{-1}$ h$^{-1}$ for 1 h on separate occasions. This established the stimulatory effects prior to studies with the receptor antagonists. Plasma SOM concentration increased approximately 3-fold in response to G-17s from 21 ± 3 to 53 ± 15 pmol l$^{-1}$ at 45 min ($P < 0·05$) (Fig. 3). CCK-8s also stimulated SOM secretion, with a peak response at 15 min increasing from 21 ± 5 to 53 ± 21 pmol l$^{-1}$ ($P < 0·05$), and declining over the remaining 45 min of the infusion (Fig. 3).

**MCR of G-17s and CCK-8s.** The mean basal levels of G-17s and CCK-8s were 46 ± 20 and 6 ± 0·3 pmol l$^{-1}$, respectively. A steady-state equilibrium was achieved by 45 min after the start of the 150 pmol kg$^{-1}$ h$^{-1}$ G-17s and CCK-8s infusions. The equilibrium level of G-17s was 313 ± 57 and 34 ± 7 pmol l$^{-1}$ for CCK-8s (Table 1). Thirty minutes after the end of the infusion plasma levels for gastrin decreased to 116 ± 29 pmol l$^{-1}$ and for CCK plasma levels decreased to basal (7 ± 3 pmol l$^{-1}$) (Table 1). The concentration of both G-17s and CCK-8s infusates were measured by RIA and the measured and calculated infusion doses were similar. The MCR of G-17s and CCK-8s was 9·6 ± 1·7 and 92·1 ± 29·7 ml kg$^{-1}$ min$^{-1}$, respectively (Table 1).

### Antagonist studies

This series of experiments used the receptor specific antagonists L-364,718 (CCK-A receptor antagonist) and L-365,260 (CCK-B/gastrin receptor antagonist) to determine the receptors involved in gastrin and CCK stimulated SOM secretion.

**Effect of antagonists L-364,718 and L-365,260 alone.** The L-364,718 bolus caused a transient increase in plasma SOM concentration from a basal value of 19 ± 4 pmol l$^{-1}$ to 47 ± 9 pmol l$^{-1}$ at 15 min ($P < 0·05$) (Fig. 4). The administration of L-365,260 also resulted in an increase in plasma SOM concentration from 22 ± 6 to 56 ± 5 pmol l$^{-1}$ at 15 min ($P < 0·05$) (Fig. 4). The vehicle alone did not change plasma SOM concentration (basal, 30 ± 4 pmol l$^{-1}$; 15 min, 34 ± 6 pmol l$^{-1}$) ($P > 0·05$) (data not shown). It is evident in Fig. 4 that although there is an immediate increase in plasma SOM levels, the partial agonist effect was transient and quantifiable. For this reason the stimulatory effect did not interfere with the protocol.

**L-364,718 or L-365,260 followed by G-17s and CCK-8s infusions.** L-364,718 and L-365,260 had different effects on G-17s-stimulated SOM secretion. As summarized by the integrated SOM outputs (Fig. 5), G-17s alone and G-17s

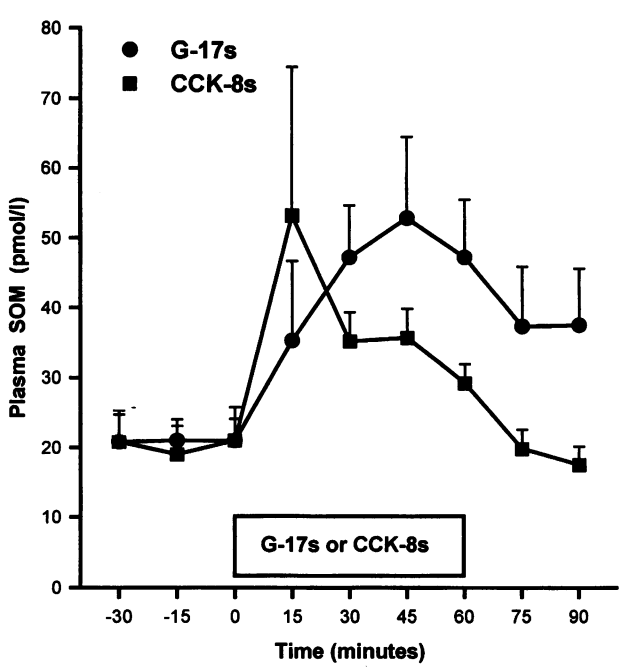


**Figure 3. Effect of I.V. infusion (150 pmol kg$^{-1}$ h$^{-1}$) of G-17s and CCK-8s on plasma SOM concentrations**
Symbols and error bars indicate means ± S.E.M., respectively, $n = 6$.

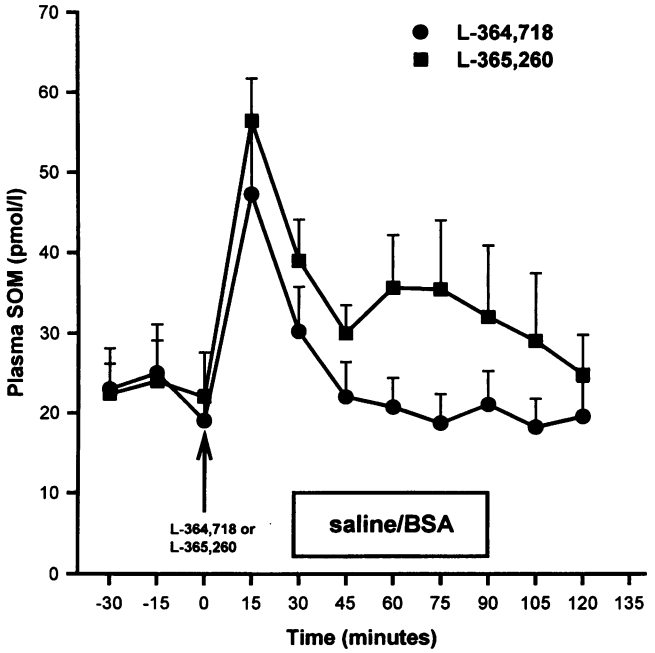


**Figure 4. Effect of L-364,718 and L-365,260 on plasma SOM concentrations**

Symbols and error bars indicate means ± S.E.M., respectively, $n = 6$ for L-364,718 and $n = 5$ for L-365,260 data.

given with a bolus of L-364,718 were not significantly different ($P > 0{\cdot}05$), with mean values of $1{\cdot}29 \pm 0{\cdot}34$ and $1{\cdot}14 \pm 0{\cdot}21$ pmol $min^{-1}$ $ml^{-1}$, respectively. In contrast, L-365,260 decreased the G-17s-stimulated SOM output from $1{\cdot}29 \pm 0{\cdot}34$ to $0{\cdot}22 \pm 0{\cdot}19$ pmol $min^{-1}$ $ml^{-1}$ ($P < 0{\cdot}05$) (Fig. 5).

L-364,718 and L-365,260 given separately both significantly suppressed CCK-8s-stimulated SOM output from a mean value of $1{\cdot}03 \pm 0{\cdot}25$ pmol $min^{-1}$ $ml^{-1}$ to $0{\cdot}19 \pm 0{\cdot}12$ (L-364,718) and $0{\cdot}24 \pm 0{\cdot}19$ pmol $min^{-1}$ $ml^{-1}$ (L-365,260), respectively ($P < 0{\cdot}05$) (Fig. 6).

**L-364,718 plus L-365,260 followed by CCK-8s infusion.** When L-364,718 and L-365,260 were administered together, CCK-8s-stimulated SOM output was significantly suppressed from a mean value of $1{\cdot}03 \pm 0{\cdot}25$ pmol $min^{-1}$ $ml^{-1}$ to $0{\cdot}32 \pm 0{\cdot}22$ pmol $min^{-1}$ $ml^{-1}$ ($P < 0{\cdot}05$). The SOM output in response to the combination of L-364,718 and L-365,260 plus CCK-8s was not significantly different from the responses of either L-364,718 plus CCK-8s and L-365,260 plus CCK-8s ($P > 0{\cdot}05$) (Fig. 6).

**Effect of antagonists L-364,718 and L-365,260 on plasma gastrin and CCK concentrations.** In contrast to the transient effect on plasma SOM concentration, L-364,718 given alone caused a sustained increase in plasma gastrin from a basal value of $39 \pm 6$ pmol $l^{-1}$ to $72 \pm 23$ pmol $l^{-1}$ ($P < 0{\cdot}05$) 30 min after the bolus injection, after which time gastrin levels continued to increase reaching a maximum value of $101 \pm 30$ pmol $l^{-1}$ at the end of the 2 h observation (Fig. 7). Similarly, L-365,260 produced an approximate 2-fold increase from a basal value of $31 \pm 5$ pmol $l^{-1}$ to $83 \pm 24$ pmol $l^{-1}$ ($P < 0{\cdot}05$) at 30 min reaching a maximum value of $126 \pm 34$ pmol $l^{-1}$ at 60 min (Fig. 7). The

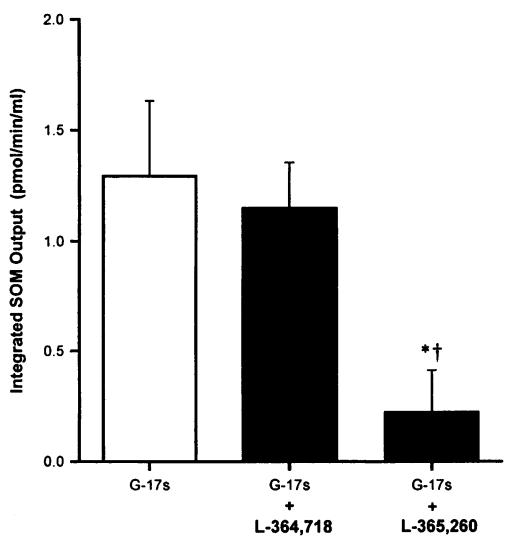


**Figure 5. Integrated SOM output in response to G-17s, G-17s plus L-364,718 and G-17s plus L-365,260**

The integrated output was calculated as the SOM output during the 60 min infusion of G-17s. Columns and error bars indicate means ± S.E.M., respectively, $n = 6$, $*P < 0{\cdot}05$ *vs.* G-17s, $\dagger P < 0{\cdot}05$ *vs.* G-17s + L-364,718.

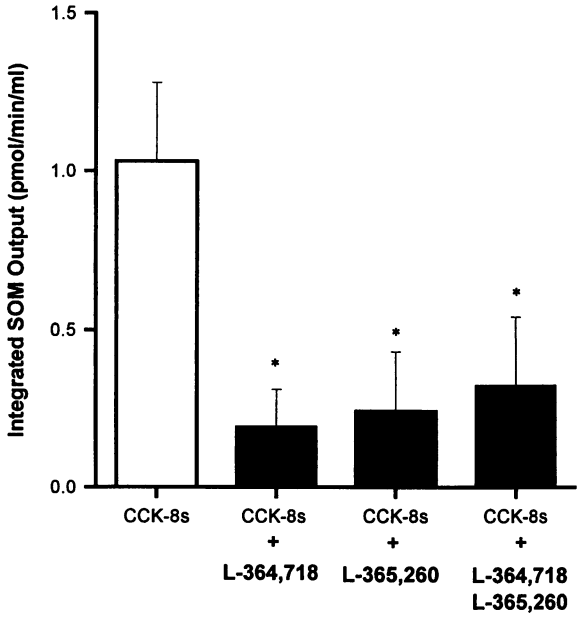


**Figure 6. Integrated SOM output (IPR) in response to CCK-8s, CCK-8s plus L-364,718, CCK-8s plus L-365,260, and CCK-8s plus L-364,718 and L-365,260**

The integrated output was calculated as the SOM output during the 60 min infusion of CCK-8s. Columns and error bars indicate means ± S.E.M., respectively, $n = 6$ except $n = 5$ for CCK-8s plus L-364,718 data, $^*P < 0{\cdot}05$ *vs.* CCK-8s.

integrated gastrin output in response to L-364,718 ($2{\cdot}92 \pm 0{\cdot}91$ pmol $min^{-1}$ $ml^{-1}$) was significantly lower than that of L-365,260 ($4{\cdot}38 \pm 1{\cdot}49$ pmol $min^{-1}$ $ml^{-1}$) ($P < 0{\cdot}05$). The vehicle alone had no effect on plasma gastrin concentration.

Plasma CCK levels increased significantly in response to L-364,718 alone from $5 \pm 1$ pmol $l^{-1}$ to a peak of $18 \pm 5$ pmol $l^{-1}$ 45 min after the bolus ($P < 0{\cdot}05$) (Fig. 8). L-365,260 also significantly increased plasma CCK levels from $5 \pm 1$ pmol $l^{-1}$ to a peak of $8 \pm 1$ pmol $l^{-1}$ at 45 min ($P < 0{\cdot}05$). The peak CCK response to L-364,718 was significantly greater than that of L-365,260 ($P < 0{\cdot}05$) (Fig. 8).

L-364,718 increased the gastric pH from a basal value of 2·7 to 5·1 units, 45 min after the bolus, after which point the gastric pH gradually declined to 3·4 units at 90 min. The changes in gastric pH with L364,718 alone or with the addition of G-17s or CCK-8s were similar. The change in gastric pH in response to L-365,260 was more modest increasing from a mean basal value of 3·1 to 3·7 units 30 min after the bolus. The gastric pH then decreased and remained close to an average value of 3·1 units. During the infusion of CCK-8s and G-17s, which followed 30 min after the administration of L-365,260, the gastric pH continued to increase to peak values of 5·7 and 4·6 units, respectively, at 90 min.

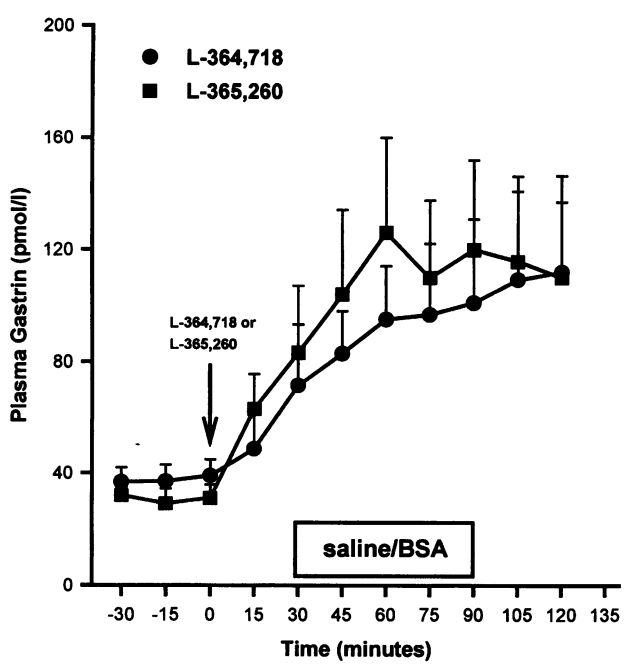


**Figure 7. Effect of L-364,718 and L-365,260 on plasma gastrin concentrations**

Symbols and error bars indicate means ± S.E.M., respectively, $n = 6$ for L-364,718 and $n = 5$ for L-365,260 data.

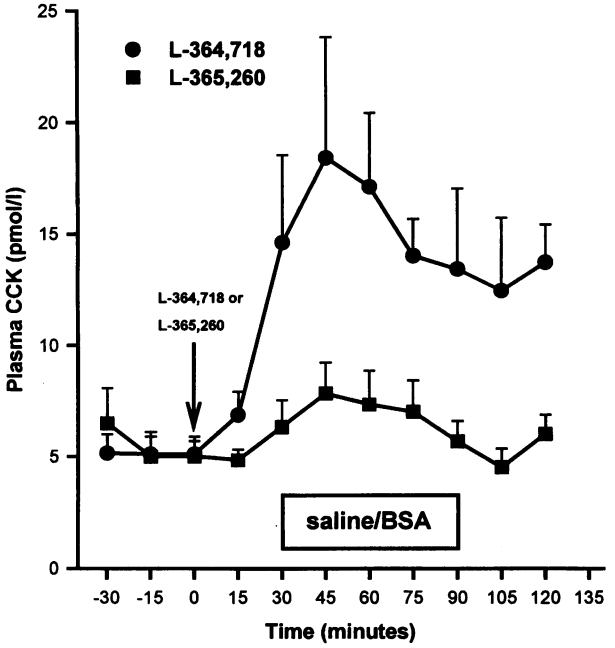


**Figure 8. Effect of L-364,718 and L-365,260 on plasma CCK concentrations**

Symbols and error bars indicate means ± S.E.M., respectively, $n = 7$ for L-364,718 and $n = 6$ for L-365,260 data.

### Reverse-phase HPLC: predominant somatostatin molecular form

The predominant SOM molecular form after both G-17s and CCK-8s stimulation was SOM-14 (Fig. 9). G-17s-stimulated SOM secretion was composed of three peaks of immunoreactivity: 83% coeluted with SOM-14, 4% coeluted with SOM-28 and a late eluting peak at approximately 17 min of 13%. The combination of L-365,260 plus G-17s decreased the amount of SOM-14 (Fig. 9). Similar results were observed in plasma collected after CCK-8s stimulation. The addition of either the type A and B receptor antagonists reduced the amount of SOM-14 in plasma. The late eluting peak (at ~17 min) observed in all elution profiles was probably a SOM precursor as described previously (Shulkes & Read, 1991).

## DISCUSSION

Despite the characterization of the CCK-A (type A) and CCK-B/gastrin (type B) receptors on the D-cell (Park, Chiba & Yamada, 1987; DelValle *et al.* 1993), the relative contribution of both the receptor subtypes as mediators of gastrin- and CCK-stimulated SOM secretion has not been investigated in *in vivo* models. Through the systematic comparison of the effects of L-364,718 (type A receptor antagonist) and L-365,260 (type B receptor antagonist) on SOM secretion, we have demonstrated that G-17s-stimulated SOM secretion is mediated by the type B receptor alone, whereas CCK-8s-stimulated SOM secretion is mediated by both the type A and B receptors. Sulphation of CCK-8 was essential for the SOM stimulatory effect of CCK but not for gastrin.

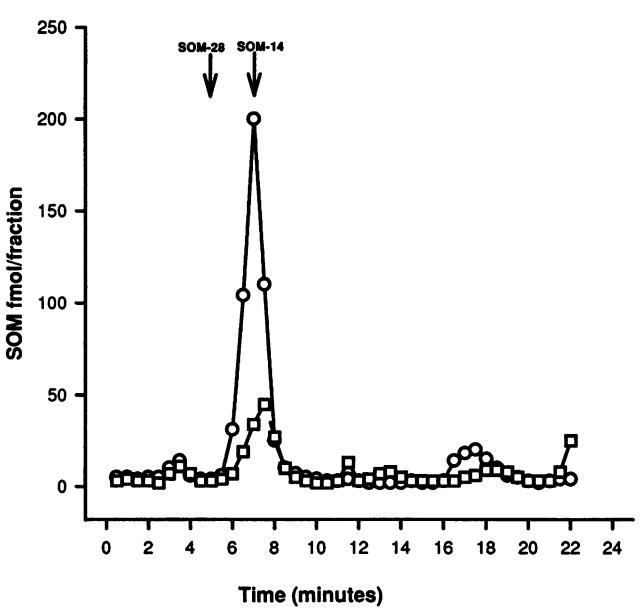


**Figure 9. HPLC of plasma after G-17s infusion alone (○), and with L-365,260 (□)**

The elution positions for SOM-14 and SOM-28 standards are shown.

The CCK-A and CCK-B/gastrin receptor antagonists administered alone behaved as partial agonists of SOM secretion causing a transient increase in plasma SOM concentration. This was unexpected as previous studies using similar effective bolus doses of 1 and 3 mg $kg^{-1}$ of L-364,718 and L-365,260, respectively, did not stimulate gastrointestinal functions including pancreatic, acid and SOM secretion either *in vivo* or *in vitro* (DelValle *et al.* 1993; Varga *et al.* 1993; Schmassmann *et al.* 1994). This is in contrast to other classes of CCK receptor antagonists such as the dipeptoid PD-136,450 which are partial agonists for gastric and pancreatic secretion (Schmassmann *et al.* 1994). Studies using L364,718 and L365,260 with mutant receptors have demonstrated that the determinants of agonist and antagonist binding to the CCK-A and -B receptor are different (Beinborn, Lee, McBride, Quinn & Kopin, 1993; Jagerschmidt, Guillaume-Rousselet, Vikland, Goudreau, Maigret & Roques, 1996) so the mechanism of the partial agonist effect of these inhibitors in the present study is not clear. Changes in amino acid sequence at transmembrane VI and VII are known to effect antagonist selectivity (Beinborn *et al.* 1993; Mantamadiotis & Baldwin, 1994; Jagerschmidt *et al.* 1996). We have sequenced part of the ovine CCK-B/gastrin receptor gene and demonstrated that homology in the VI and VII transmembrane domains between sheep and man is 95% (G. S. Baldwin, unpublished observation). Importantly, human, rat and sheep have valine at position 349, and histidine at position 376 (numbering for human) and these are the critical amino acids that determine specificity for L364,718 and L365,260 (Beinborn *et al.* 1993; Jagerschmidt *et al.* 1996).

The CCK-B/gastrin receptor antagonist L-365,260 significantly inhibited the stimulatory effect of G-17s on SOM secretion showing that gastrin stimulates SOM secretion by activating the CCK-B/gastrin receptor. In contrast the CCK-A receptor antagonist L-364,718 did not suppress G-17s-stimulated SOM secretion. Our results confirm that the CCK-A receptor is selective for CCK-8 since G-17-stimulated SOM secretion was not affected by the type A receptor antagonist (DelValle *et al.* 1993; Silvente-Poirot *et al.* 1993).

CCK-8s-stimulated SOM secretion was suppressed by L-364,718 confirming previous reports in other species (Lloyd *et al.* 1992*b*; DelValle *et al.* 1993). The type B receptor antagonist L-365,260 also suppressed the stimulatory effect of CCK-8s on SOM secretion indicating that the CCK-B/gastrin receptor also has a role as a mediator in CCK-8s-stimulated SOM. The inhibition of CCK-8s-stimulated SOM secretion was similar for both antagonists and the combination of antagonists was not different from the antagonist administered alone. This suggests that CCK-8s binds to both the type A and type B receptors and blocking either of the receptors results in suppression of the overall response.

As in man (Schmidt *et al.* 1994), the plasma concentrations of CCK in sheep are much less than those of gastrin. The present work suggests that part of this difference is that the MCR of CCK ($92{\cdot}1 \pm 29{\cdot}7$ ml $kg^{-1}$ $min^{-1}$) is higher than gastrin ($9{\cdot}6 \pm 1{\cdot}7$ ml $kg^{-1}$ $min^{-1}$). Cuber, Bernard, Gibard & Chayvaille (1989) reported a similar value for CCK of 135 pmol $kg^{-1}$ $h^{-1}$ in the pig and there is a general consensus that the MCR of gastrin is around 10 pmol $kg^{-1}$ $min^{-1}$ (Pauwels, Dockray, Walker & Marcus, 1985; Dockray, Hamer, Evans, Varro & Dimaline, 1991; Ciccotosto & Shulkes, 1992). There are few studies in which the MCR of gastrin and CCK have been compared. Calculation of the MCR from the plasma CCK and gastrin levels presented in the report by Schmidt *et al.* (1994) show that the clearance of CCK was 4-fold higher than that of gastrin. However, unlike the current study the concentrations of the infusates were not measured. This difference between gastrin and CCK MCR has important implications in assessing potency in that the same dose of the two peptides has a vastly different effect on plasma levels (Table 1). Indeed, based on plasma levels achieved, CCK is more potent than gastrin in stimulating SOM, and this is supported by the earlier peak in SOM concentration following the infusion of CCK-8s (Fig. 3). The effective dose of CCK and gastrin used (150 pmol $kg^{-1}$ $h^{-1}$) was similar or less than that reported by others to examine the release of SOM into the circulation (Lloyd *et al.* 1992*b*; Greenberg, Fung & Pokol-Daniel, 1993). Recent studies in man used lower doses but no release of SOM into the circulation was detected (Verhulst *et al.* 1994; Schmidt *et al.* 1994).

SOM-14 was the predominant molecular form in the circulation following CCK or gastrin stimulation. More than 80% of the recovered immunoreactivity was SOM-14 with the remainder being SOM-28 and small amounts of late eluting peaks. The small proportion of SOM-28 in the plasma is consistent with the released SOM originating from the stomach (abomasum) since the antrum contains more than 95% SOM-14 and the fundus between 80–90% of SOM-14, while the intestine has a larger proportion of SOM-28 (Shulkes, 1994). The chromatography confirmed that the antagonists reduced the secretion of SOM-14.

Unlike the transient stimulatory effect of the antagonists on SOM secretion, both antagonists caused a stimulation of gastrin secretion which was sustained throughout the 2 h recording period. The mechanism for the increase in plasma gastrin is unclear. It may be the result of the decrease in gastric acidity initiated by the transient increase in SOM. Parietal and G-cells are differentially sensitive to SOM, since doses that effectively inhibit acid secretion may have no effect on gastrin (Vatn, Schrumpf, Hansen & Myren, 1977; Colturi, Unger & Feldman, 1984). Because of the sustained stimulatory effect on gastrin secretion, a more likely explanation is that the antagonists have blocked the tonic inhibitory effect of CCK-stimulated SOM secretion on gastrin. Another possibility is that the increase in gastrin reflects the removal of an autoinhibition of the G-cell.

Both the CCK-A and CCK-B receptor antagonists increased gastric pH with the type A receptor antagonist having a

somewhat greater effect. In contrast, in man the type A receptor antagonist has no effect on basal gastric acidity (Schmidt *et al.* 1994). The mechanism for the present observation of the increase in gastric pH following the type A receptor antagonist could relate to the transient increase in SOM which would inhibit the parietal cell. The increase in gastric pH following the type B receptor antagonist would in addition involve the direct inhibition of the CCK-B/gastrin receptor on the parietal cell. Based on the present investigation the stimulatory effect of type A and B receptor antagonists on gastrin secretion involves two mechanisms: a blockade of CCK-A and CCK-B receptor mediated paracrine SOM secretion and an inhibition of gastric acid secretion.

Plasma CCK levels were elevated in response to the type A receptor antagonist. In humans, the type A receptor antagonist potentiates meal-stimulated CCK release but has no effect on basal plasma CCK concentration. The increase in circulating CCK has been explained by a bile and/or enzyme-mediated feedback loop in which the antagonist blocks enzyme secretion (Konturek *et al.* 1993). A further explanation is that the type A receptor antagonist inhibits CCK-stimulated SOM secretion allowing an unopposed increase in plasma CCK concentration. The type B receptor antagonist increased plasma CCK concentration although to a lesser extent than the A antagonist. This agrees with our finding that CCK-8-stimulated SOM secretion is mediated by both the type A and B receptors.

CCK stimulates SOM secretion from both antral and fundic cells in culture (Soll *et al.* 1985; Buchan, Curtis & Meloche, 1990) and in the whole animal (Rouiller, Schusdziarra, Harris & Unger, 1980; Lloyd *et al.* 1992*b*; Greenberg *et al.* 1993). SOM is thought to be the final mediator of the enterogastrone effect of CCK on post-prandial gastric acid secretion (Lloyd *et al.* 1992*b*). The inhibitory effect of type A receptor antagonists on CCK-stimulated SOM secretion and the reversal by these antagonists of nutrient induced inhibition of gastric acid secretion (Lloyd *et al.* 1992*b*; Konturek *et al.* 1993; Greenberg *et al.* 1993) has led to the assumption that only the type A receptor is involved in this response. The present study questions this assumption by demonstrating that in addition to activation of the type A receptor, activation of the type B receptor by CCK also stimulates SOM. Our findings are supported by recent data demonstrating the presence of type B receptors on D-cells from both antrum and fundus (Song *et al.* 1996).

In summary, CCK-8s stimulates SOM secretion through both the CCK-A and -B receptors while G17-s stimulates SOM secretion by the CCK-B/gastrin receptor alone. The present study further elucidates the role of CCK-8s as a negative regulator of gastrin as either type A or B receptor blockade resulted in an increase in circulating gastrin. A number of investigators have proposed that the excess gastrin and gastric acid secretion associated with *Helicobacter pylori* infection and duodenal ulcer disease is the result of loss of inhibitory drive of SOM and a failure of CCK to stimulate SOM (Moss, Legon, Bishop, Polak & Calam, 1992; Konturek *et al.* 1993; El-Omar, Penman, Ardill, Chittajallu, Howie & McColl, 1995). The increase in gastric secretion is further exaggerated in *Helicobacter pylori* positive patients who have active duodenal ulcers suggesting that impaired inhibitory control may not be uniform in the fundus and antrum (El-Omar *et al.* 1995). It is known that SOM is regulated differentially from these two regions (Sandvik, Dimaline, Forster, Evans & Dockray, 1993) and it will be important to determine whether there are equivalent effects of gastrin and CCK on SOM secretion in the acid and non-acid secretory regions of the stomach.


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